Ecological Indicators 108 (2020) 105687

Contents lists available at ScienceDirect

Ecological Indicators

journal homepage: www.elsevier.com/locate/ecolind

Original Articles

Potential impacts of lanthanum and yttrium through embryotoxicity assays with *Crassostrea gigas*

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ARTICLE INFO

Keywords:
Rare earth elements
Toxicity
Crassostrea gigas
Embryo development

ABSTRACT

In the last decades, the production of electronic devices, which relies on rare earth elements (REE) such as yttrium (Y) and lanthanum (La), has increased exponentially, a pattern that should continue in the future. Besides the lack of raw materials, the growing generation of electronic waste (E-waste) and the REEs direct or indirect discharge into the environment raises worldwide concern. Mostly, given the scarcity of data about their toxicity to marine biota. In this study, a standardized toxicity test on oyster *Crassostrea gigas* embryonic development was performed to assess the impacts of La and Y. Lanthanum exerted significantly higher toxicity than Y, with median effect dose concentrations (EC_{50}) of 6.7 to 36.1 $\mu g\,L^{-1}$ (24 and 48 h) for La; and 147 to 221.9 $\mu g\,L^{-1}$ (24 and 48 h) for Y. Higher toxicity of La was likely related to higher bioavailability of free ionic form (La^{3+}). Comparison of toxicity thresholds with literature indicated that La is among the most toxic compounds to *C. gigas* embryos, while Y was ranked among compounds with intermediate toxicity. Overall, this work brings new insights into the potential implications of the widespread use of high-tech products, if appropriate management measures are not taken.

1. Introduction

The massification of technology in human life, particularly the widespread use of electronic devices such as smartphones, computers or tablets in recent decades is inevitably associated with a growing consumption of rare earth elements (REE). This group of chemical elements, comprising scandium, yttrium and the 15 lanthanoids of the periodic table, has a set of unique properties (e.g. Para magnetism, conductivity, fluorescence, etc.) that make them crucial in an expanding range of strategic applications (Zepf, 2013).

Yttrium (Y) and lanthanum (La) are common elements in low energy light bulbs, along with europium, cerium (Ce), terbium, argon and mercury (Tan et al., 2015). Other phosphors and luminescence applications of Y and La include displays and light emission diodes (i.e. LEDs), and are also used as colouring and decolouring agents in glass (De Lima, 2015). Lanthanum compounds are used as a petroleum cracking catalyst (Kulaksiz and Bau, 2011), as adsorbents to control phosphorus loads in water (Chen et al., 2016; Gibbs et al., 2011), while Lanthanum Yttrium Germanate (LaYGe) finds application in alternative energy technologies as electrolyte material for solid oxide fuel cells (Kendrick and Slater, 2008).

According to the most recent available data, the global demand for REE is rising at a rate of 3.7–8.6% annually (Tan et al., 2015) – corresponding to more than 130 000 tons in 2018 (Zhou et al., 2017), which entails several concerns related with the scarcity of raw materials and with the environmental impacts associated with increased mining exploration and of wastewaters from industrial production (Jacinto et al., 2018).

Another key issue is the production of waste electrical and electronic equipment, commonly known as E-waste, which is presently considered to be one of the waste streams that grows faster, at 3–5% annually (EUROSTAT, 2018). In 2016, the amount of E-waste generated worldwide has reached 44.7 million metric tons, equivalent to almost 4500 Eiffel towers (Baldé et al., 2017).

Due to their chemical similarities, REE coexist in nature in their most common oxidation state of +3 (Zepf, 2013), with abundances in the Earth crust varying between 0.28 $\mu g\,g^{-1}$ of thulium and 66 $\mu g\,g^{-1}$ of Ce (Wang and Liang, 2015). In pristine waters, REE are found in low concentrations, in the range of $ng\,L^{-1}$ – $\mu g\,L^{-1}$ (Kabata-Pendias and Mukherjee, 2007; Sultan and Shazili, 2009), with discrete differences

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https://doi.org/10.1016/j.ecolind.2019.105687
Received 27 April 2019; Received in revised form 15 August 2019; Accepted 29 August 2019
Available online 13 September 2019
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among elements, except occasional anomalies of Ce and Europium (Eu) (Kulaksiz and Bau, 2011). Migaszewski and Gałuszka (2015) reviewed the abundance of some REEs including La considering the terrestrial and aquatic compartments. In river water, the concentration of La ranged between 19.7 ng L^{-1} (Mississippi River) and 74.0 ng L^{-1} (Amazon River) reaching higher concentrations in wastewater like ore mine (USA) and coal mine (China) effluent that is 80.4 μg L^{-1} and 7.77 μg L^{-1}, respectively. In EU stream sediment, the La concentration was up to 41 mg kg^{-1}. Soil and crustal rocks ranged between 25.9 and 57 mg Kg^{-1}.

Reports of anthropogenic enrichment of REE in waters are becoming more and more frequent, particularly for La, Y, samarium and gadolinium (Hissler et al., 2014; Kulaksiz and Bau, 2011; Olías et al., 2005; Tepe et al., 2014) with a few studies showing REE accumulation in biota (Li et al., 2013; Merschel and Bau, 2015; Qiang et al., 1994; Weltje et al., 2002). However, eco-toxicity and potential impacts to marine ecosystems are not known.

Several recent studies have reviewed what is known about the toxicity of rare earth elements, including health impacts on humans - both occupational and environmental exposures (Pagano et al., 2015; Rim et al., 2013; Zhang et al., 2000), and in plants and animals (Gonzalez et al., 2014; Herrmann et al., 2016; Rogowska et al., 2018; Tyler, 2004). The published literature points to the need for more studies to adequately estimate REE impacts, and to establish regulatory limits on emissions and concentrations of these elements in the environment (Herrmann et al., 2016). Apparently contradictory effects were attributed to REE, for example positive and negative effects on plant growth (Agathokleous et al., 2018), and antioxidant/prooxidant effects on *Vicia faba* L. seedlings (Wang et al., 2012), which may be explained by the hormesis phenomenon, i.e. a dual concentration-effect trend. Furthermore, ecotoxicity studies are almost confined to Ce and La (Oral et al., 2010), with only few works addressing the comparison of REEs toxicities (Pagano et al., 2015, 2012; Tai et al., 2010). Also, relatively less information exists about the impacts of REE on aquatic organisms (González et al., 2015), particularly regarding marine organisms, which seems contradictory given that most effluents are discharged into water bodies - rivers, estuaries and coastal environments (Gonzalez et al., 2014).

This work aimed to produce information about the potential toxicity of two rare earth elements (Y and La) to aquatic organisms, by means of standard embryotoxicity assays with *Crassostrea gigas*. Comparison between element toxicities, and type of effect on organism development were addressed.

2. Material & methods

2.1. Embryotoxicity assay

Toxicity of Y and La were assessed based on the sensitivity of oyster (*Crassostrea gigas*) embryo-larval development following standardized embryotoxicity protocols (His et al., 1997; ISO 17244, 2015; Leverett and Thain, 2013). All material necessary to perform the bioassays was cleaned and sterilized prior experiments. Artificial seawater, with salinity 33 (reference salinity), was prepared 3 days before the experiments by dissolution of Tropic Marine® Sea Salt in ultrapure water (Leverett and Thain, 2013), for further use in exposure media preparation and spawning/fertilization procedures. Seawater was filtered (0.2 μm) through cellulose acetate filters (Millipore) using a vacuum filtration unit (pH = 8.19, total alkalinity of 2.0 meq kg^{-1}).

Analytical grade standard solutions of Y and La (Alfa Aesar Specpure®, plasma standard solutions, 1000 mg L^{-1}, Y_2O_3 and La_2O_3 in 5% HNO_3) were used to spike seawater at different exposure concentrations of 2.5, 5.0, 10, 20, 40 and 160 μg L^{-1} of both Y and La in separate. Exposure concentrations were selected not only considering their potential environmental reliability (i.e. relative maximum concentrations of both Y and La in brackish water effluents (< 1 μg L^{-1} to 50 μg L^{-1}, (Sultan and Shazili, 2009)); but also to allow the identification of the median effect concentration (EC50).

Exposure assays were conducted in 24-well sterile capped microplates, where each well (3 mL) was considered an independent exposure vessel. All solutions were distributed in separate microplates in order to: i) expose developing embryos to all concentrations during two separate timeframes (24 and 48 h post fertilization); ii) determine Y and La effective concentrations in independent vessels in the absence of embryos. Each tested condition was replicated in triplicate for embryo exposures, and in quadruplicate for effective dose determination analysis. Copper ($Cu(NO_3)_2$) at concentrations of 3, 6, 12, 18 and 30 μg L^{-1} was used as positive control (reference toxicant) (Libralato et al., 2009). Microplates were incubated at 24 ± 1 °C overnight, before spawning induction took place to stabilize testing media at the exposure temperature.

Mature *C. gigas* oysters were purchased from Guernsey Farms (UK), and allowed to rest in flowing seawater (15 °C) for 1 day, at the laboratory. Spawning was induced by thermal stimulation, by changing oysters from seawater baths set at 18 and 28 °C in 30 min consecutive intervals. Gamete quality (oocyte shape and sperm motility) was evaluated using a microscopy, and the best males (n = 4) and females (n = 3) selected for gamete emission. Oocytes from each female were collected in separate beakers, the resulting oocyte suspensions were posteriorly filtered through a 100 μm nylon mesh and mixed in 500 mL final volume (salinity 33, 24 °C). Male gametes were collected in separate beakers, filtered through a 45 μm nylon mesh into a mixed suspension, and left to activate for 20 min. Oocyte suspensions were fertilized by adding approximately 10^6 spermatozoa to 1 oocyte cell. Fertilization success was identified by microscopy.

Fertilized zygotes were posteriorly transferred to microplates containing the exposure media in order to obtain approx. 200 embryos per well. Microplates with the exposure media (0, 2.5, 5.0, 10, 20, 40 and 160 μg L^{-1} of Y or La) destined for embryo exposures and for REE quantification, as well as the positive control (3, 6, 12, 18 and 30 μg L^{-1} of Cu^{+2}), were incubated in the dark for 24 and 48 h (24 ± 1 °C).

After incubations, embryo-larval development was stopped by adding buffered formalin (4%). Analysis followed visual inspection of 100 embryos per well, under an inverted microscope, and characterization of the relative frequencies of different types of development (D-shape, pre-D, protruded mantle, kidney shape, indented shell and dead larvae, Fig. 1) according to His et al. (1997).

2.2. Rare earth elements (REE) quantification

Spiked seawater contained in microplate wells destined to REE quantification was collected after incubations and analysed via ICP-MS (Thermo X-Series) (Table 1). Speciation analysis was performed for each REE to infer on the predominant chemical forms in exposure media, using VisualMinteq software (version 3.1). For this, seawater composition was based on Atkinson and Bingmann (1997) – Table 2, measured values of pH (8.19), total alkalinity determined by potentiometric titration (Gran, 1952) of 2001 ± 5.8 μmol kg^{-1} (mean ± std, n = 3) and temperature (24 °C) of the exposure of incubation. Because results from speciation analysis revealed to be insensitive to exposure concentration, results provided are valid for all exposure conditions.

2.3. Data analysis and statistics

Results were analysed in terms of percentage of abnormal larvae (including pre-D and other malformations). Toxicity threshold values for Y and La were determined based on median effect concentrations (EC_{50s}) for each REE, after different times of exposure (24 and 48 h). For this, data on abnormal development observed at each exposure concentration were corrected for the effects observed in the respective

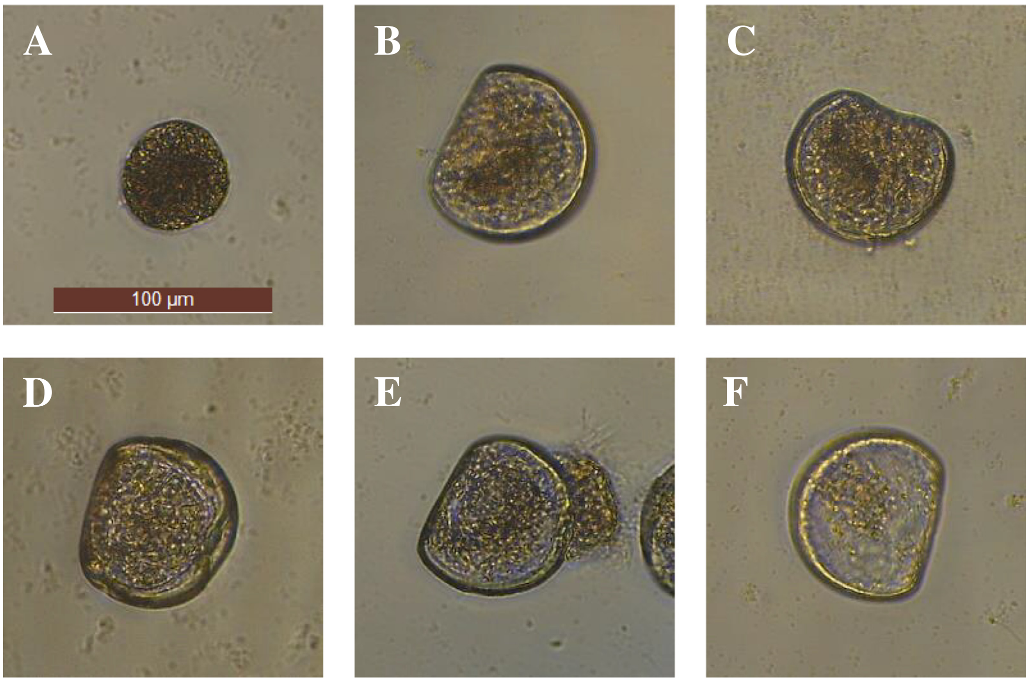

Fig. 1. Photographic record of some *C. gigas* larval development types, taken after exposure to Y or La: A) pre-D; B) D-shape; C) kidney shape; D) indented shell; E) protruded mantle; and F) dead larvae.

negative control with the Abbott's formula (American Society for Testing and Materials, 2004). Data were submitted to non-linear regression analysis using GraphPad Prism 6.01. Effective Y and La concentrations retrieved from ICP-MS for each condition were used for toxicity calculations, after lognormal transformation. The best fit concentration-response curve values allowed to estimate the median effect concentrations (EC_{50}s) and respective 95% confidence intervals for each element tested, after 24 or 48 h. Analysis of variance among EC_{50} values was performed using one-way ANOVA, followed by Tukey's multiple comparisons tests, based on values of Log(EC_{50}) and the associated standard error returned by the software for each estimated curve (GraphPad Prism version 6.01). D'Agostino and Pearson omnibus test was used to verify the underlying assumptions of normality.

Table 2
Elemental composition of synthetic sea salt Tropic Marin ® (adapted from Atkinson and Bingmann (1997)).

Major Cations ($mmol\,kg^{-1}$)		Major Anions ($mmol\,kg^{-1}$)		Nutrients ($\mu mol\,kg^{-1}$)		Trace ($\mu mol\,kg^{-1}$)			
Na^{+}	442	Cl^{-}	497	PO_4:P	1.20	Li	29	Cu	1.9
K^{+}	9.1	SO_4^{-2}	21	NO_3:N	2.20	Si	14	Zn	0.55
Mg^{+2}	46	TCO_2	1.10	NH_4:N	0.55	Mo	2.5	Mn	0.7
Ca^{+2}	9.1	TB	0.36	SiO_3:Si	3.2	Ba	0.32	Fe	0.24
Sr^{+1}	0.08			DOP:P	0	V	2.8	Cd	0.24
				DON:N	5.5	Ni	1.7	Pb	2.3
				TOC:C	32	Cr	7.6	Co	1.3
						Al	230	Ag	2.7
								Ti	0.62

3. Results & discussion

Data obtained from the embryotoxicity assays allowed to fit the concentration-response curves for Cu^{2+} (reference toxicant), La and Y, and to calculate the EC_{50} for each element after 24 or 48 h via non-linear regression analysis (Fig. 2). Median effect dose concentrations (EC_{50}) in $\mu g\,L^{-1}$ and the respective 95% confidence intervals were: 19.9 (19.2–20.5) for Cu^{2+}; 6.7 (5.6–7.9) for La (24 h); 36.1 (30.4–42.8) for La (48 h); 147.1 (121.4–178.2) for Y (24 h); and 221.9 (169.3–291.0) for Y (48 h). The validity of the embryotoxicity experiments was checked by comparison of the results obtained in the negative and positive controls, to those reported in the literature. In the negative controls, frequencies of well-developed D-shape larvae of all replicates exceeded 82%, within the acceptable frequencies of normal developed larvae (D-shape) ≥ 80% (His et al., 1999). The toxicity of the reference toxicant was interpolated at an EC_{50} of 19.9 (19.2–20.6) $\mu g\,L^{-1}$ of Cu^{2+}, a value located in the upper range of toxicity threshold previously reported for *C. gigas* (4.6–28.7 $\mu g\,L^{-1}$) according to (Libralato et al., 2010), thus validating the oyster embryo-larvae bioassay.

Considering the results obtained for La and Y exposures, embryo-larval development was most affected by La, presenting the lowest EC_{50s} observed (6.7 $\mu g\,L^{-1}$ 24 h; 36.1 $\mu g\,L^{-1}$ 48 h). Among the few existing data on REE toxicity to marine invertebrate early development, studies on the embryotoxicity of La to sea urchin *Paracentrotus lividus* have been carried out. After assessing *P. lividus* embryo-larval defects after exposure to La for 72 h, Oral et al. (2010) reported EC_{50} for La at 833.4 $\mu g\,L^{-1}$; while Pagano et al. (2016) reported EC_{50} of 92.5 $\mu g\,L^{-1}$, both sets of values higher than the ones obtained in the present study.

Oyster embryo-larval development was less sensitive to Y than to La, given the lower extent of embryotoxicity observed, that resulted in higher EC_{50s} (147.1 $\mu g\,L^{-1}$, 24 h; 221.9 $\mu g\,L^{-1}$, 48 h) compared to La

Table 1
Analytical concentrations of Lanthanum (La) and Yttrium (Y) measured by ICP-MS for each condition (mean ± std, n = 4).

Condition	CTL (0.0)	C1 (2.5)	C2 (5.0)	C3 (10)	C4 (20)	C5 (40)	C7 (160)
Y ($\mu g\,L^{-1}$)	< 0.5	2.5 ± 0.07	5.5 ± 0.4	11 ± 1.4	22 ± 1.4	45 ± Ag2.8	158 ± 3.2
La ($\mu g\,L^{-1}$)	< 0.2	1.8 ± 0.07	3.7 ± 0.07	7.7 ± 0.07	17 ± 0.00	34 ± 0Ti.7	133 ± 1.3

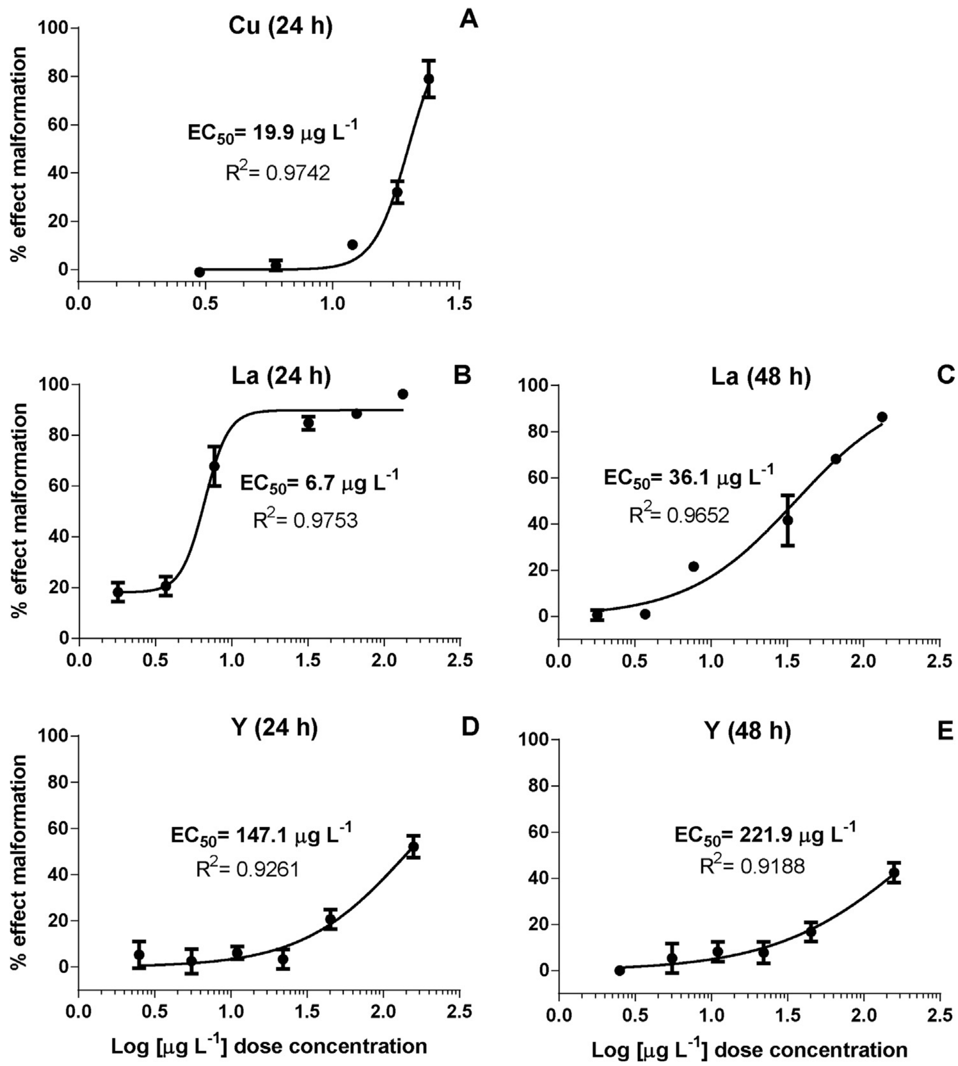

Fig. 2. Dose response curves of *Crassostrea gigas* embryo-larvae exposed to Cu after 24 h post fertilization (A); Lanthanum after 24 (B) and 48 h (C); and Yttrium after 24 (D) and 48 h (E). Effective exposure concentrations are in Log [$\mu g\,L^{-1}$]. Median effect dose concentrations (EC_{50}) and goodness of fit (R^2) are provided for each element.

Table 3
Toxicity of different types of pollutants considering available data on *Crassostrea gigas* embryo-larval bioassays. Median effect concentrations (EC_{50}) and the extent of exposure are provided for each reference.

	Compound	EC_{50} ($\mu g\,L^{-1}$)	Exposure time (h)	References
Metal(loid)s	Cu	4.6–28.7	24	Libralato et al. (2010)
	Pb	12.5		Mai et al. (2012)
	Hg	12.3		His et al. (1999)
	Cd	212.3		Mai et al. (2012)
	As	452.0		Moreira et al. (2018)
Rare earth elements	La	6.6, 36.1	24, 48	This study
	Y	147.1, 221.9		
Pharmaceuticals	Sertraline	67.1	36	Di Poi et al. (2014)
	Amitriptyline	185.7		
Pesticides	Glyphosate	28.3	48	Mottier et al. (2013)
	AMPA	40.6		
Herbicides	Dinoterbe	72.2	24	His et al. (1999)
Nanoplastics	NH2-50	150.0	36	Tallec et al. (2018)
	COOH-50	11600.0		

(Fig. 2). In contrast, Pagano et al. (20160 showed that both La and Y induced a similar extent of developmental defects in sea urchin *P. lividus* embryos, having reported no significant differences between EC_{50} of La: 92.5 (13.5–630.6) $\mu g\,L^{-1}$, and of Y: 70.9 (25.5–197.2) $\mu g\,L^{-1}$ (72 h post fertilization). Concerning Y, the EC_{50} determined in the present study (147.1 $\mu g\,L^{-1}$, 24 h; 221.9 $\mu g\,L^{-1}$, 48 h), and the respective 95% confidence intervals (121.4–178.2, 24 h; 169.3–291.0, 48 h) overlap with those determined by Pagano et al. (2016) (25.5–197.2 $\mu g\,L^{-1}$) for sea urchin *P. lividus*, thus indicating similar toxicity threshold values for Y in both *C. gigas* and *P. lividus* embryos.

The inconsistency of comparability of toxicity thresholds (i.e. EC_{50} of La in *C. gigas* lower than in *P. lividus*; and similar EC_{50} of Y between taxa) is not surprising, knowing that intraspecific differences of toxicity thresholds are not uncommon due to several factors (e.g. physiological status; moment of contact with toxicant; seawater characteristics; methodological variations) (Morroni et al., 2018), therefore higher discrepancy should be expected between interspecific toxicity thresholds. In fact, under similar methodological conditions oyster embryo-larval development can be more sensitive than sea urchin ones, as demonstrated for particular pollutants (His et al., 1999; Tsunemasa et al., 2012), and could be one of the reasons why oyster embryos presented higher sensitivity to La in the present study, compared to that observed by Pagano et al. (2016) with sea urchins. To provide a more specific perspective of the toxicity of La and Y to *C. gigas* embryo-larval development, a review on EC_{50} values for different types of contaminants determined under similar exposure conditions, were summarized in Table 3. Looking at EC_{50} values obtained in the present study and those reported by other authors, La could be ranked among the most toxic compounds to *C. gigas* embryos, alongside metals (Cu, Pb, Hg) (His et al., 1999; Mai et al., 2012; Pagano et al., 2016) and pesticides (glyphosate) (Mottier et al., 2013), thus warranting further attention under the eminence of increased La use and production worldwide. Concerning Y, EC_{50} could be ranked among contaminants of intermediate toxicity such as pharmaceuticals (Amitriptyline) (Di Poi et al., 2014), nanoplastics (NH_2-50) (Tallec et al., 2018) or cadmium (Mai et al., 2012) (Table 3).

Significant differences were observed among median effect dose concentration (EC_{50}) values, namely La presented significantly lower EC_{50} than Y considering both exposure times, but also EC_{50s} was significantly lower after 24 h than after 48 h for both REE (Fig. 3).

Firstly, higher embryotoxicity of La compared to Y observed, could at least be partially due to differences on chemical speciation of each REE occurring in solution, knowing that physical and chemical characteristics of La and Y render different chemical behaviour in seawater (Bau et al., 1996; Kawabe et al., 1991; Quinn et al., 2004). For instance, heavy REEs (e.g. Y) possess relatively smaller ionic radii and therefore present higher stability constants towards naturally relevant complexes in comparison to light REEs (e.g. La) (Zhenggui et al., 2001). Indeed, speciation analysis showed important differences in the prevalent forms of La and Y in the exposure media. Particularly, the predominant forms of La were $LaCO_3^+$ (43.5% total concentration), followed by $LaSO_4^+$ (22.1%) and La^{3+} (15.8%); while the predominant forms of Y were $Y(CO_3)^+$ (47.4%) and $Y(CO_3)^{2-}$ (40.3%), followed by YPO_4 (3.4%) and Y^{3+} (3.1%) (Table 4). According to Moermond et al. (2001), in seawater depleted from organic matter, La is likely to complexate with carbonate ions, or to occur in the trivalent form (La^{3+}). In line with this, speciation analysis indicated the predominance of a La carbonate complexes ($LaCO_3^+$), and the La ion La^{3+}, despite the high percentage of $LaSO_4^+$. The relatively high proportion of La^{3+} in solution (15.8%) versus Y (3.1%), could represent one of the main reasons explaining higher toxicity of La to oyster embryo larvae, given that the trivalent forms should be the most bioavailable and cytotoxic forms of both La (Das et al., 1988) and Y (Jakubek et al., 2009). This hypothesis gains further strength given that the mechanism of action of both REEs studied are similar. At the cellular level, La (as La^{3+}) is thought to compete for binding sites with calcium ions (Ca^{2+}), thus inhibiting Ca channels and interacting with membrane associated enzymes (Jakubek et al., 2009). Similarly, the interaction of trivalent Y has been shown to inhibit Ca channels (Jakubek et al., 2009). Furthermore, Y has been shown to be the most potent inhibitor of T-type Ca channels among several trivalent cations including La (Beedle et al., 2002). Therefore, higher toxicity of La observed should be attributed to higher bioavailability, rather than related to differences in cellular mechanisms of action itself. The interference of both these REEs with Ca homeostasis, and consequent interruption of Ca-dependent signaling cascades could be responsible for the embryonic malformations observed (Morroni et al., 2018).

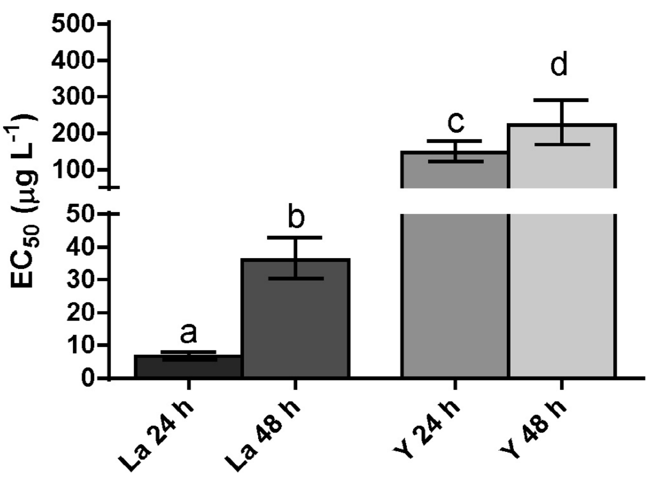

Fig. 3. Median effect dose concentrations (EC_{50}) and respective 95% confidence intervals of Lanthanum (La) and Yttrium (Y) to *Crassostrea gigas* embryo-larvae after 24 and 48 h post fertilization. Significant differences ($p \leq 0.05$) among EC_{50} values are represented with different letters.

Table 4
Speciation analysis of Lanthanum (La) and Yttrium (Y) in exposure media inferred with VisualMinteq 3.1. Seawater constitution based on (Atkinson and Bingmann, 1997), and measured pH (8.19), total alkalinity (2001 µmol kg^{-1}) and Temperature (24 °C) of the exposure media.

La		Y	
Species name	% of total concentration	Species name	% of total concentration
$LaCO_3^+$	43.5	$Y(CO_3)^+$	47.4
$LaSO_4^+$	22.1	$Y(CO_3)^{2-}$	40.3
La^{3+}	15.8	YPO_4(aq)	3.4
$La(CO_3)^{2-}$	9.7	Y^{3+}	3.1
$La(SO_4)^{2-}$	3.8	YSO_4^+	3.1
$LaCl^{2+}$	3.7	YOH^{2+}	1.4
$LaOH^{2+}$	0.7	YCl^{2+}	0.8
$LaHCO_3^{2+}$	0.7	$Y(SO_4)^{2-}$	0.6

Finally, the influence of time of exposure on toxicity threshold determination observed for each REE (significant differences between EC_{50} between 24 and 48 h exposures) was likely related to the interplay between natural developing rates and embryonic development blockage/delay induced by these elements. This was particularly evident for La, supported by observations of delayed embryo-larval development, with relatively high percentages of Pre-D embryos observed in a dose dependent manner (Fig. 4). Other types of contaminants have shown to induce developmental delay during marine invertebrate early development, including in *C. gigas* embryos exposed to As (Moreira et al., 2018), and *P. lividus* embryos exposed to Cd, Cu, Pb and Zn (Morroni et al., 2018), despite that both these studies demonstrated that the arrested development is not necessarily permanent. Interestingly, results obtained in the present study suggest that the arresting effect of La was permanent, given that the percentages of Pre-D larvae were similar considering 24 and 48 h exposures, particularly at the highest La concentrations tested. Hence, the difference between EC_{50} determined for La between 24 and 48 h was related to a shift of the dose response curve to the right due to a lower effect noted at the lowest concentrations, mostly because higher frequencies of D-shape larvae were observed after 48 h at the lowest La concentrations. In contrast, oyster embryos exposed to Y did not present the same extent of developmental arrest, with no apparent differences on the frequency of Pre-D embryos among tested concentrations, except for those exposed

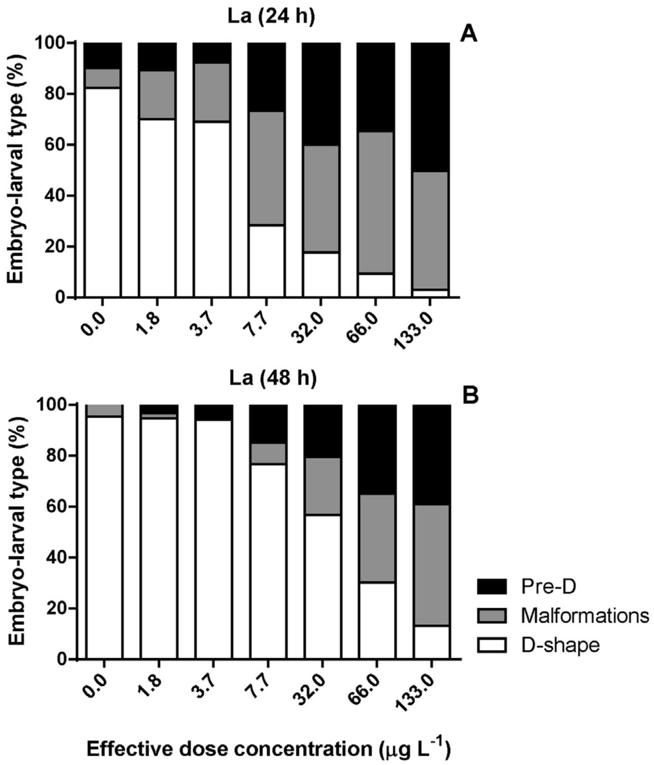

Fig. 4. *Crassostrea gigas* embryo-larval types (D-shape, Malformations, Pre-D) after exposure to increasing concentrations of Lanthanum (La), during 24 (A) and 48 h (B) post fertilization.

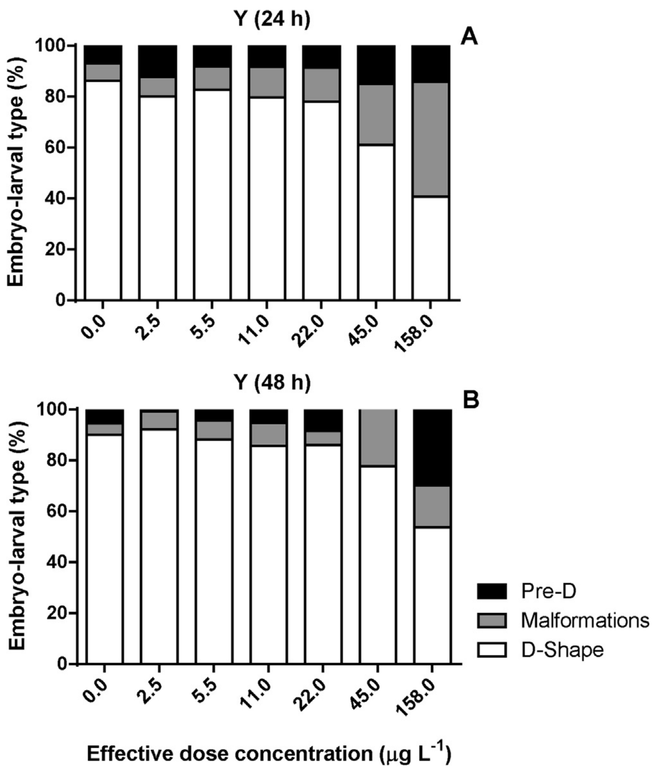

Fig. 5. *Crassostrea gigas* embryo-larval types (D-shape, Malformations, Pre-D) after exposure to increasing concentrations of Yttrium (Y), during 24 (A) and 48 h (B) post fertilization.

to the highest Y concentration for 48 h (Fig. 5). Similarly to La, differences between EC_{50} of Y between 24 and 48 h exposures were mostly related to a shift of the dose response curve towards the highest Y concentrations, given the overall higher frequencies of D-shape larvae observed in all conditions tested. These findings highlight the usefulness of using different times of exposure criteria when employing the *C. gigas* embryo bioassay, by providing a deeper insight on the effects of embryo development blockage/delay, thus providing a wider estimation of toxicity threshold values.

4. Concluding remarks

Given the scarcity of information regarding REEs toxicity to marine biota, the present study aimed to provide new insights on the effects of different REEs (La and Y), by use of the well-established *C. gigas* embryo-larval development assay. Results obtained allowed to determine median effect dose concentrations (EC_{50}) after different times of exposure (24 and 48 h), and to compare with those from several other compounds, thus providing important evidence on the potential effects of these elements considering the increasing widespread use in the industry. Under the bioassay conditions employed, La exerted higher embryotoxicity than Y, that was likely related to higher bioavailability of free ionic form (La^{3+}) inferred by speciation analysis. Comparison of toxicity thresholds of these REEs with those from other types of pollutants indicated that La is among the most toxic compounds to *C. gigas* embryos, while Y was ranked among compounds with intermediate toxicity. Further research is needed to gain insightful knowledge about La and Y speciation in natural systems. This study brings new data on toxicity threshold values for two important REEs, suggesting that increased concentrations of La in seawater may pose a hazardous threat to important shellfish resources such as oysters, thus providing relevant information for stakeholders and/or policy makers for better management policies for REEs in the upcoming future.

Acknowledgments

Anthony Moreira benefited from PhD grant SFRH/BD/93107/2013, funded by FCT – Fundação para a Ciência e a Tecnologia. Bruno Henriques benefited from a Research position funded by national funds (OE), through FCT – Fundação para a Ciência e a Tecnologia, I.P., in the scope of the framework contract foreseen in the numbers 4, 5 and 6 of the article 23, of the Decree-Law 57/2016, of August 29, changed by Law 57/2017, of July 19. Rosa Freitas benefited from a Research position funded by Integrated Programme of SR&TD “Smart Valorization of Endogenous Marine Biological Resources Under a Changing Climate” (reference Centro-01-0145-FEDER-000018), co-funded by Centro 2020 program, Portugal 2020, European Union, through the European Regional Development Fund. Thanks are also due for the financial support to CESAM (UID/AMB/50017), to FCT/MEC through national funds, and the co-funding by the FEDER, within the PT2020 Partnership Agreement and Compete 2020.

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